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# Sensitivity of *Escherichia albertii*, a Potential Food-Borne Pathogen, to Food Preservation Treatments▽

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***Escherichia albertii* is a potential food-borne pathogen because of its documented ability to cause diarrheal disease by producing attachment and effacement lesions. Its tolerances to heat (56°C), acid (pH 3.0), and pressure (500 MPa [5 min]) were evaluated and found to be significantly less than those of wild-type *E. coli* O157:H7.**

*Escherichia albertii* is a potential food-borne pathogen that may contribute to the burden of food-borne illness in the United States. Previously classified as *Hafnia alvei* with the ability to produce intimin (2, 11), it was reported to cause diarrheal disease in six children with accompanying symptoms of vomiting, mild dehydration, fever, and abdominal distention (2, 3). Five isolates of *H. alvei* from six children suffering from diarrhea in Bangladesh were reclassified as *E. albertii* based on their biochemical properties, genetic homology to *Escherichia coli* and *Shigella flexneri*, and unique lipopolysaccharide elements (1, 9, 10, 12). *E. albertii* isolates produce intimin, a protein that allows enteropathogenic *E. coli* and enterohemorrhagic *E. coli* to form attachment and effacement lesions on human intestinal epithelial cells. Commercial systems have misidentified *E. albertii* as *Yersinia ruckeri*, *Salmonella enterica* serovar Enterica, *H. alvei*, or *E. coli* (1, 19). The lack of biochemical identification of these strains has limited investigation into the incidence of *E. albertii* in foods; however, an *eae*-positive isolate of *H. alvei* was isolated from minced meat in Norway (14), possibly indicating that this *H. alvei* strain was actually *E. albertii*. *E. albertii* could possibly contribute to the estimated 62,000,000 cases of food-borne illnesses and 3,200 deaths in the United States that have an unknown etiological origin (15). The potential virulence of *E. albertii* and its possible presence in several foodstuffs led to our evaluation of its tolerance to interventions used in the food industry.

*E. albertii* 9194, 10457, 10790, 12502, and 19982 and the closely related *Shigella boydii* 12032 have been described previously (11); *E. coli* O157:H7 strain 52 (ATCC 43895; nalidixic acid and rifampin resistant) and an *rpoS*-deficient mutant of strain 52 (strain 55; *rpoS*::pRR10), along with *S. flexneri*, were also described previously (4, 6). Heat tolerance was measured by determining thermal decimal reduction times at 56°C ($D_{56°C}$ values) as described by Sharma and Beuchat (18); methods for determination of acid tolerance (pH 3.0 for 2 h at 37°C) were described by Bhagwat and Bhagwat (4). Stationary-phase cultures were treated in a PT-1 hydrostatic press (Avure Technologies, Kent, WA) at 500 MPa for 1 or 5 min at 22°C. Treated cells were enumerated on nonselective media. Among *E. albertii* strains, strain 19982 showed the most tolerance to heat and pressure treatments, while strain 9194 displayed the greatest acid tolerance.

$D_{56°C}$ values of *E. albertii* strains 19982 and 10457 were significantly ($P \leq 0.05$) greater than those of strains 9194, 10790, and 12502 (Table 1). *E. albertii* strains are closely related to *S. boydii* 12032, as determined by multilocus sequence typing of housekeeping genes, differing in 0.72% of nucleotide sites examined (11). However, $D_{56°C}$ values for *S. boydii* 12032 were significantly higher than those of *E. albertii*.

No *E. albertii* strain tested had a higher $D_{56°C}$ value than any wild-type *E. coli* O157:H7 strain examined, but all strains had $D_{56°C}$ values similar to those of the *rpoS*-deficient *E. coli* O157:H7 55 strain. Variability in wild-type *rpoS* alleles and other stress response genes of *E. albertii* strains may also ac-

TABLE 1. $D_{56°C}$ values for *E. albertii*, *E. coli* O157:H7, and *Shigella* sp. strains

| Pathogen and strain | $D_{56°C}$ ± SE (min)[a] |
|---|---|
| *E. albertii* | |
| 9194 | 2.9 ± 0.1 C |
| 10457 | 5.0 ± 0.0 B |
| 10790 | 2.7 ± 0.3 C |
| 12502 | 2.7 ± 0.1 C |
| 19982 | 5.5 ± 0.0 B |
| *E. coli* O157:H7 | |
| 52 | 7.4 ± 0.7 A |
| 55 | 4.0 ± 0.4 BC |
| *S. boydii* 12032 | 8.9 ± 2.8 A |
| *S. flexneri* | 4.7 ± 1.0 B |

[a] Mean values ($n = 3$) that are not followed by the same letter indicate significant ($P < 0.05$) differences.

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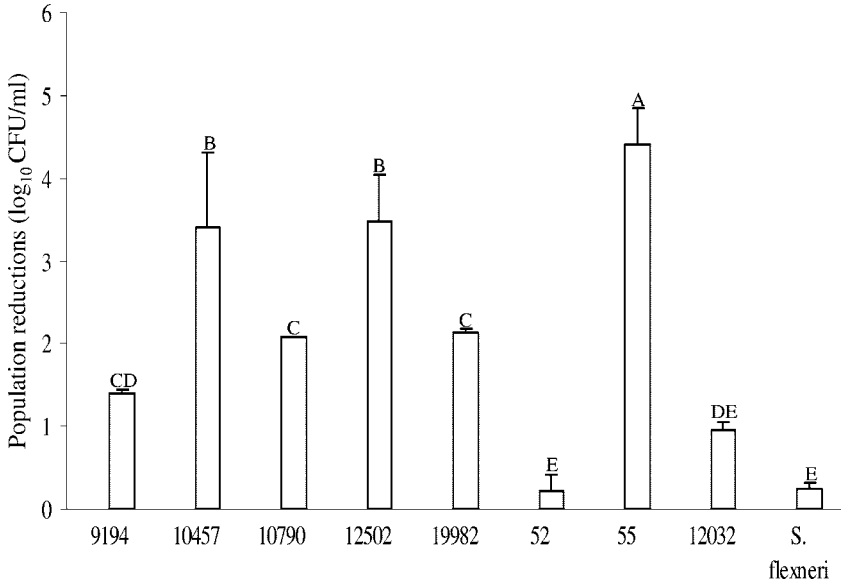


FIG. 1. Acid tolerance, as measured by population reduction ($\log_{10}$ CFU/ml), of *E. albertii*, *E. coli* O157:H7, and *Shigella* spp. after 2 h at pH 3.0 for 37°C. Strains are represented as follows: *E. albertii*, 9194, 10457, 10790, 12502, and 19982; *E. coli* O157:H7, 52 and 55; *S. boydii*, 12032; and *S. flexneri*. Letters above error bars that are not the same indicate significant ($P \leq 0.05$) differences in population reductions (open bars) of bacterial strains ($n = 2$).

count for differences in the $D_{56°C}$ values of these strains. In a previous study examining enterohemorrhagic *E. coli*, the acid, heat, and alkali tolerances of strains were influenced by the wild-type *rpoS* allele present in a strain (6).

*E. albertii* strains 9194, 10790, and 19982 displayed significantly greater acid tolerances than strains 10457 and 12502 (Fig. 1). Strain 9194 had acid tolerance similar to that of *S. boydii* 12032. No strain of *E. albertii* displayed more acid tolerance than wild-type *E. coli* O157:H7 strain 52. Of all the strains tested, *rpoS*-deficient *E. coli* O157:H7 strain 55 was the

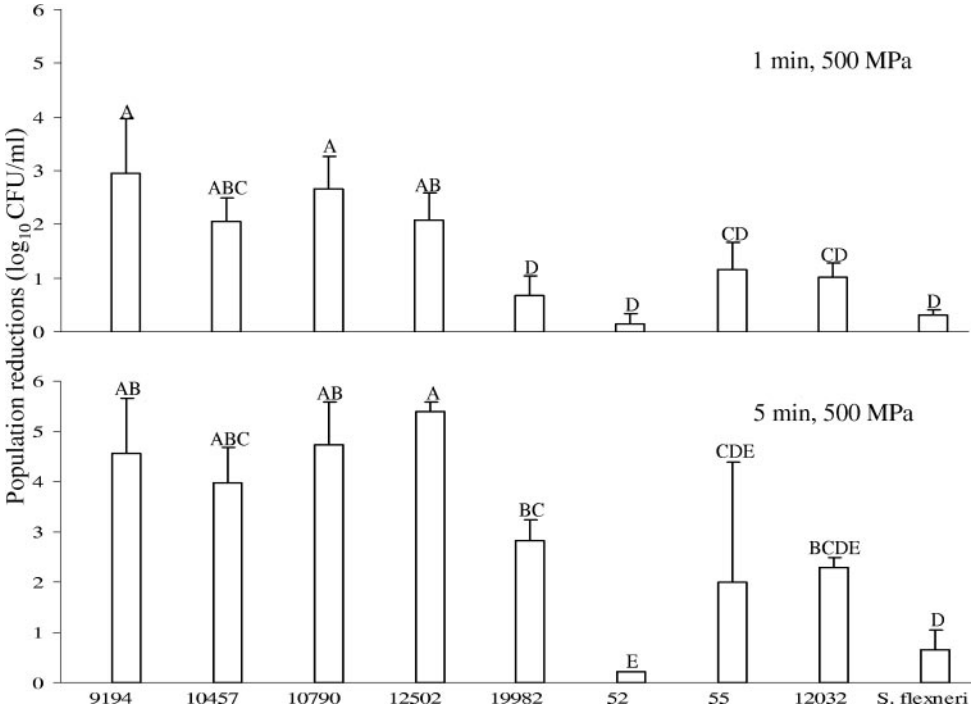


FIG. 2. Pressure tolerance, as measured by reductions ($\log_{10}$ CFU/ml) in populations of *E. albertii*, *E. coli* O157:H7, and *Shigella* spp. after treatment at 500 MPa and 22°C for 1 min and 5 min. Strains are represented as follows: *E. albertii*, 9194, 10457, 10790, 12502, and 19982; *E. coli* O157:H7, 52 and 55; *S. boydii*, 12032; and *S. flexneri*. For each of the indicated treatment times, different letters above error bars indicate significant ($P \leq 0.05$) differences in population reductions (open bars) of bacterial strains treated with hydrostatic pressure ($n = 2$).

least acid tolerant. The diversity of acid tolerance responses in strains of *E. albertii* suggests that the acid response may be based on the functional heterogeneity of stress response genes. Experiments conducted in our work evaluated AR-1, the acid resistance mechanism induced when bacterial cells are oxidatively metabolizing carbon-containing nutrients (8, 13, 16). Previous work has shown that *rpoS*-deficient *E. coli* O157:H7 strains do not induce AR1 as effectively as wild-type strains and are more sensitive to oxidative acidic stress (5).

*E. albertii* strains showed significantly lower pressure tolerances than wild-type *E. coli* O157:H7 strain 52 after 5 min (Fig. 2). *E. albertii* strain 19982 showed greater pressure tolerance than strain 12502 after 5 min. There were no significant differences in pressure tolerances between other *E. albertii* strains and strains 19982 and 12502. Significant differences were not observed in pressure tolerances of wild-type and *rpoS*-deficient strains of *E. coli* O157:H7, a result which is not in agreement with previous work which reported a reduction of $>2$ $\log_{10}$ CFU/ml for the *rpoS*-deficient compared to wild-type strain results (17). Our work shows a similar reduction in populations of wild-type and *rpoS E. coli* O157:H7 but lacks statistical significance. The pressure tolerance of *S. flexneri* and *E. coli* O157:H7 is in agreement with previous studies which found that *S. flexneri* and *E. coli* O157:H7 had the highest decimal reduction pressure value (7). Even after pressure treatment at 500 MPa for 10 min at 22°C, tolerances of *E. albertii* strains were less than those of wild-type *E. coli* O157:H7 and *S. flexneri* strains (data not shown).

This study was the first to evaluate the tolerances of strains of *E. albertii* for food preservation processes used in industry. Strains of *E. albertii* were not more tolerant of these stresses than wild-type *E. coli* O157:H7, indicating that measures used to kill *E. coli* O157:H7 should be sufficient to inactivate *E. albertii*. Variations in tolerances of *E. albertii* strains to heat, acid, and pressure were observed. Culture methods to distinguish *E. albertii* strains from *E. coli* O157:H7 should be developed to evaluate the incidence of *E. albertii* in foods.

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